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(71) Applicant: COSTAR CORPORATION [US/US]; One Alewife Center, Cambridge, MA 02140 (US).

(72) Inventors: LYMAN, George ; P.O. Box 1267, Kennebunkport, ME 04046 (US). NARDONE, Joanne ; 50 Deer Grass Lane, Concord, MA 01742 (US).

(74) Agent: GATES, Edward, R.; Wolf, Greenfield & Sacks, 600 Atlantic Avenue, Boston, MA 02210 (US).

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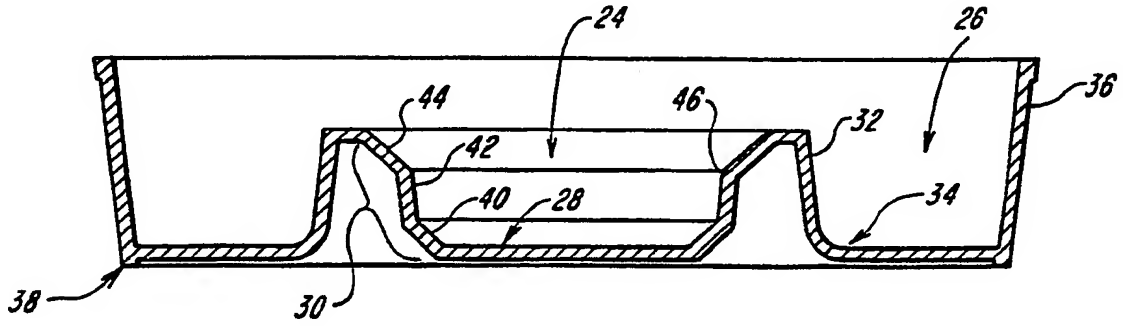
(54) Title: DISH FOR IN VITRO FERTILIZATION



(57) Abstract

The invention provides a novel dish (22) for use in conducting *in vitro* fertilization procedures. The dish has a first well (24) including a flat transparent bottom (28) and a surrounding side wall (30) constructed and arranged to channel an egg or embryo toward the bottom wall (28) to eliminate surfaces capable of trapping an egg or embryo, and to facilitate access to all sides of an egg or embryo positioned at the periphery of the bottom wall (28). A second well (26) is attached adjacent to the first well (24) for containing a volume of humidification fluid. The first well (24) may include a volumetric marking preferably formed by the junction (46) of two side wall portions (42, 44).

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DISH FOR IN VITRO FERTILIZATION

This invention relates in general to tissue culture dishes and in particular to a dish useful in connection with in vitro fertilization procedures.

Background of the Invention

In vitro fertilization is a procedure involving the fertilization of an egg in a controlled environment, such as in a culture dish. In vitro fertilization may be used to study the dynamics of conception and early embryonic development. More recently it has been used as a substitute for natural conception when natural conception has not been successful or is impossible. An egg previously removed from a female and sperm previously collected from a male are contacted in a vessel under controlled conditions to promote fertilization of the egg and early stage development of an embryo. The embryo subsequently may be implanted by a medical procedure into the uterus of a female where it is allowed to further develop as a fetus to full term as in a normal pregnancy.

In vitro fertilization is a relatively new science which has resulted in the use of equipment originally designed for organ culture applications. An organ culture dish (Fig. 1) has a culture well 10 including a horizontally disposed shelf 12. The culture well 10 is surrounded by an annular humidification well 14. In a typical procedure, one ml of medium is placed in the culture well 10 for nourishing the egg and later the embryo. One ml of medium fills the culture well 10 of the organ culture dish to the horizontal shelf 12. Approximately 4 ml of medium is placed in the humidification well 14 and provides humidification equilibrium when the dish is covered and placed in the incubator. A single egg which has been surgically removed from the follicle of a female is placed in the medium in the culture well 10. The dish then is covered with a loose fitting lid designed to allow controlled gas exchange and is placed in an incubator for approximately 24 hours. Then, approximately 100,000 sperm are added to the culture well 10 containing the single egg 16. The dish again is covered and returned to the incubator for another 24 hours. Next, the bottom surface of the culture well 10 is examined under lowest magnification with an inverted microscope to locate the egg 16. After the egg is located, it is

examined under greater magnification to determine whether fertilization has occurred (i.e. pronuclei formation). If fertilized, the egg is placed in a fresh dish, is incubated for an additional 24 hours, and then is implanted into the uterus of the female.

When using the organ culture dish of the prior art, certain problems occur due to its structure. Because one ml of medium fills the central culture well 10 to the level of the horizontal shelf 12, when moving the dish, for example, to the incubator, medium often splashes onto the horizontal shelf 12. Should the egg or sperm be deposited on this shelf, death may result from lack of nourishment or lack of moisture. The size and shape of the culture well 10 also causes problems and inconvenience. The lowest stage of magnification on most inverted microscopes covers a field smaller than the diameter of the bottom wall 20 of the culture well 10. Because the egg or embryo may be found anywhere on the bottom wall 20, it is sometimes necessary to move the organ culturing dish around to locate the egg or embryo. Because the side wall 18 of the culture well 10 extends substantially perpendicularly from the bottom wall 20 of the central culturing well 10, viewing the egg and accessing the egg are hampered when the egg is located at the junction between the side wall 18 and bottom wall 20. As shown in Fig.

2, to access an egg positioned at this junction may require vertical orientation of a pipette, bringing the user's hand directly over the culture well 10, which is unacceptable according to conventional practice.

These and other drawbacks are overcome by the present invention which provides a tissue culturing dish specifically adapted for in vitro fertilization procedures. The dish has a central culturing well having an optically transparent bottom wall surrounded by a side wall for containing a volume of incubation fluid. The dish also has an annular humidification well attached to and surrounding the central well. The surrounding side wall is constructed and arranged to channel an egg toward the bottom wall of the central well and to facilitate access to an egg positioned at the junction between the bottom wall and the side wall. Preferably, the lower portion of the side wall is integral with the bottom wall and extends at an obtuse angle from the bottom wall, most preferably at about 135°. It is also preferable that the top portion of the surrounding side wall extend at an obtuse angle to the bottom wall, preferably at about 135°. In a most preferred embodiment, the side wall is formed of three portions, a lower portion that extends upwardly at an angle of about 135° from the

periphery of the bottom wall, a central portion that extends upwardly from the top of the lower portion at a steeper angle than the lower portion and an upper portion that extends outwardly from the top of the central portion at an angle of about 135° to the bottom wall. The bottom wall preferably is not larger than the field of a conventional inverted microscope set at its lowest stage of magnification, and most preferably is circular with a diameter of about .64 inches.

The central well may be provided with a volumetric mark for defining a fixed-volume reaction chamber in the central well. The portion of the side wall outside of this reaction chamber and immediately adjacent to the volumetric marking means is non-horizontal, thereby channeling liquid contacting this non-horizontal portion into the reaction chamber.

The foregoing design allows the user to more efficiently and conveniently conduct an <u>in vitro</u> fertilization procedure. The invention eliminates the shelf of the prior art dishes and also ensures that an egg or embryo is channeled into the nutrient medium and onto the microscopic field of vision. The dish also facilitates the manipulation of the egg or embryo by allowing better access to the periphery of the bottom wall of the central well.

Brief Description of the Drawings

Fig. 1 shows an organ tissue culture dish of the prior art;

Fig. 2 is a schematic illustration of the dish of Fig. 1 in use;

Fig. 3 is a perspective view of the tissue culture dish of the invention;

Fig. 4 is a cross-sectional view of the tissue culture dish of Fig. 3; and

Fig. 5 is a schematic illustration of the dish of Fig. 3 in use.

Detailed Description of the Drawings

The preferred tissue culture dish 22 of the invention is shown in Fig. 3 and includes two wells, a central, circular, well 24 and an annular well 26 surrounding the central well 24. The central well serves as a vessel for containing an egg, sperm and embryo, while the annular well 26 serves as a humidification chamber during incubation. The central well 24 and annular well 26 are integrally formed and are attached to one another at their adjacent walls.

The central well 24 is formed of a bottom wall 28 and a surrounding, funnel-shaped side wall 30.

The annular well 26 is substantially U-shaped in cross-section, having an inner wall 32 an annular bottom wall 34 and a circumferential outer wall 36. The inner wall 32 of the annular well is adjacent to and attached at its upper end to the side wall 30. The circumferential outer wall 36 is taller than the side wall 30 and inner wall 32 and provides a supporting surface for a lid (not shown), thereby supporting the lid above the inner wall 32 and side wall 30 to ensure atmospheric communication between the humidification annular well 26 and the central well 24. The lower edge 38 of the outer wall 36 extends beneath the plane defined by the bottom walls 28, 34 and serves to elevate the bottom wall 28 of the central well 24 from a surface upon which the dish 22 is placed to prevent scratching of the bottom wall 28. This protruding lower edge 38 also may be constructed and arranged to fit into a mating stacking ring of a conventional tissue culture lid (not shown).

The funnel-shaped side wall 30 of the central well 24 has three distinct portions, a lower portion 40, a middle portion 42 and an upper portion 44. The lower portion 40 is integral with and extends from the periphery of the bottom wall at an angle of about 135° to facilitate access to all surfaces of an egg or embryo located close to the periphery of

the bottom wall 28. While an angle as great as 135° is not required, it is preferred. The slope of this lower portion, however, should be substantial enough to ensure that an egg or embryo will be channeled into the field of vision defined by the circumference of the bottom wall 28.

The middle portion 42 of the side wall 30 extends from the top end of the lower portion 40 and is oriented at an angle close to vertical with respect to the bottom wall 28. It also acts to channel the egg or embryo toward the bottom wall 28 of the central well 24.

The upper portion 44 of the side wall 30 slopes upwardly and outwardly from the top of the middle portion 42, preferably at an angle of approximately 135° to the bottom wall. The angle of the upper portion further facilitates access to an egg or embryo positioned close to the periphery of the bottom wall 28. As may be seen with reference to Fig. 5, if the angled upper portion 44 were eliminated and replaced with a substantially vertical extension of the middle portion 42 (indicated by dotted line) then a pipetce would have to be oriented at an angle closer to vertical. Such an orientation would bring the user's hand over the dish which is not desirable.

The junction 46 of the upper portion 44 and middle portion 42 of the side wall 30 has been located to act as a volumetric marking defining a fixed-volume growth chamber formed by the middle portion 42, lower portion 40 and bottom wall 28 of the dish. Preferably, this reaction chamber holds one milliliter of fluid. The central well 24, therefore, may be filled with one milliliter of medium without using a graduated pipette by introducing medium into the central well 24 until the meniscus reaches the junction 46. Since the upper portion 44 of the side wall 30 is outside the reaction chamber and slopes upwardly from the junction 46, any material splashed from the reaction chamber against this wall will be channeled back into the reaction chamber.

In the preferred embodiment, the tissue culture dish 22 of the invention may be manufactured as a single piece by injection molding and preferably is formed of medical or tissue culture grade polystyrene. The areas of the mold corresponding to the bottom wall 28 of the central well 24 are optically polished to ensure a transparent surface. The bottom wall also preferably is flat to further enhance viewing with an inverted microscope. The dish may be tissue culture treated according to various procedures

known to those skilled in the art to enhance culturing and further may be radiation sterilized before use.

The dimensions of the dish of the most preferred embodiment are as follows. The thickness of the central bottom wall 28, side wall 30, inner wall 32 and annular bottom wall 34 is about .04 inches. The thickness of the outer wall 36 of the annular well 26 is about .025 inches. The diameter of the central bottom wall 28 is about .64 inches, while the diameter of the central well 24 at the junction is about .77 inches. In the preferred embodiment, the height from the bottom wall to the junction is .175 inches and the height from the bottom wall to the top of the upper portion 44 of the side wall 30 is about .30 inches.

While a specific design has been shown in the preferred embodiment, the invention is not intended to be limited by this specific design. The purposes of the design of the side wall are: to prevent surfaces that tend to trap cells, to provide surfaces that channel cells into the nutrient medium and onto the microscopic field of vision; to provide a volumetric marking; and to facilitate access to an egg or embryo positioned close to the periphery of the bottom wall. Consequently, the absolute number of constituent wall portions and the angles at which they intersect may vary consistent with these goals.

It should be understood that the foregoing description of the invention is intended merely to be illustrative and that other embodiments, modifications and equivalents thereof may be apparent to those skilled in the art without departing from the scope or spirit of the invention. Having thus described our invention, what we desire to claim and have secured by letters patent is:

CLAIMS

1. A dish for conducting in vitro fertilization procedures comprising,

a first well having a flat transparent bottom and surrounding side wall means for containing a volume of incubation fluid, said surrounding side wall means being constructed and arranged to channel an egg toward the bottom wall, to eliminate surfaces capable of trapping an egg, and to facilitate access to all sides of an egg positioned at the periphery of the bottom wall, and

a second well attached adjacent to said first well for containing a volume of humidification fluid.

2. A dish as claimed in claim 1 further comprising a volumetric marking means for defining a fixed-volume growth chamber within the first well, the side wall including a non-horizontal portion immediately adjacent to the volumetric marking means and sloping upwardly and outwardly from the marking means for channeling liquid contacting said non-horizontal portion into the growth chamber.

3. A dish as claimed in claim 1 wherein the first well is a central circular well and the second well is an annular well substantially U-shaped in cross-section and surrounding said first well.

4. A dish for conducting in vitro fertilization procedures as claimed in claim 1 wherein the first well has a substantially circular bottom wall with a diameter of about .64 inches.

5. A dish for conducting in vitro fertilization procedures as claimed in claim 1 wherein the side wall means defines a growth chamber and a wall portion sloping upwardly and outwardly from said growth chamber.

6. A dish for conducting in vitro fertilization procedures as defined in claim 1 wherein the dish is made of polystyrene injection molded as a single piece from a mold at least a portion of which is optically polished.

7. A dish as claimed in claim 1 wherein said surrounding side wall means includes a lower wall portion formed integral with and extending at an angle of about 135° from the bottom wall.

8. A dish as claimed in claim 7 further comprising a middle portion that extends upwardly from the top of the lower portion at a steeper angle than the lower portion, and an upper portion that extends upwardly and outwardly from the top of the central portion at an angle of about 135° from the bottom wall.

9. A dish as claimed in claim 7 wherein the dish is injection molded as a single piece of medical grade polystyrene.

10. A dish for conducting in vitro fertilization procedures comprising,

a first well having a flat transparent bottom and surrounding side wall means for containing a volume of incubation fluid,

a second well attached adjacent to said first well for containing a volume of humidification fluid, and

volumetric marking means on the side wall means for defining a fixed-volume growth chamber within said first well, the side wall means including a non-horizontal portion immediately adjacent to the volumetric marking means for channeling liquid contacting said non-horizontal portion into the growth chamber.

11. A dish as claimed in claim 10 wherein said non-horizontal portion slopes upwardly and outwardly from said volumetric marking means.

12. A dish as claimed in claim 11 wherein the marking means is a junction of two differently sloping side wall portions.

13. A dish as claimed in claim 12 wherein the first well has a circular bottom wall having a diameter of about .64 inches.

14. A dish as claimed in claim 11 wherein the side wall means has a lower portion integral with and extending from the periphery of the bottom wall at an angle of about 135° to the bottom wall.

15. A dish as claimed in claim 14 wherein the first well has a circular bottom wall having a diameter of about .64 inches.

16. A dish as claimed in claim 11 wherein the first well is funnel-shaped and the second well is substantially U-shaped in cross-section and surrounds the funnel-shaped well.

17. A dish as claimed in claim 12 wherein the side wall means has a lower portion integral with and extending from the periphery of the bottom wall at an angle of about 135° to the bottom wall.

18. A tissue culture dish comprising,

a central well having a flat bottom wall and a surrounding side wall, said side wall having a lower portion that extends upwardly at an angle of approximately 135° from the periphery of the bottom

wall, a central portion that extends upwardly from the top of the lower portion at a steeper angle than the lower portion and an upper portion that extends upwardly and outwardly from the top of the central portion at an angle of approximately 135° to the bottom well,

and an annular well surrounding the central well and having a bottom wall and inner and outer side walls, said inner side wall being connected to the surrounding side wall of the central well.

19. A tissue culture dish as defined in claim 18 wherein,

the diameter of the flat bottom wall is not larger than the field of a conventional inverted microscope set at its lowest stage of magnification.

20. A tissue culture dish as defined in claim 18 wherein,

the inner side wall of the annular well has a top end connected to the top of the upper portion of the side wall of the central well.

21. A tissue culture dish as defined in claim 19 wherein,

the inner side wall of the annular well has a top end connected to the top of the upper portion of the side wall of the central well.

22. A dish for conducting in vitro fertilization procedures and adapted to be used with an inverted microscope comprising,

a central well having a flat bottom wall and a surrounding side wall, said side wall having a lower portion that extends upwardly at an obtuse angle from the periphery of the bottom wall, a middle portion that extends upwardly from the top of the lower portion at a steeper angle than the lower portion and an upper portion that extends upwardly and outwardly from the top of the middle portion,

and an annular well surrounding the central well and having a bottom wall and inner and outer side walls, said inner side wall being connected to the surrounding side wall of the central well.

23. A dish for conducting in vitro fertilization procedures as defined in claim 22 wherein,

the upper portion of the side wall of the central well is inclined at an angle of approximately 135° to the bottom wall.

24. A dish for conducting in vitro fertilization procedures as defined in claim 22 wherein the lower portion of the side wall of the central well is inclined at an angle of approximately 135° to the bottom wall.

25. A dish for conducting in vitro fertilization procedures as defined in claim 22 wherein the dish is made of polystyrene injection molded as a single piece from a mold at least a portion of which is optically polished.

26. A dish for conducting in vitro fertilization procedures as defined in claim 11 wherein the dish is made of polystyrene injection molded as a single piece from a mold at least a portion of which is optically polished.

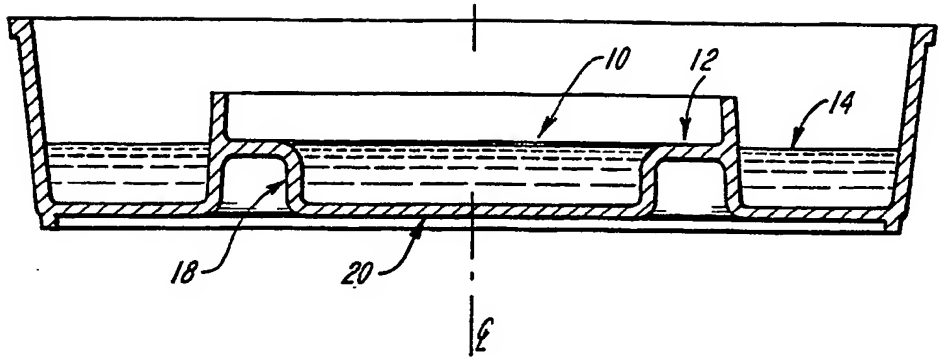

FIG. 1
(PRIOR ART)

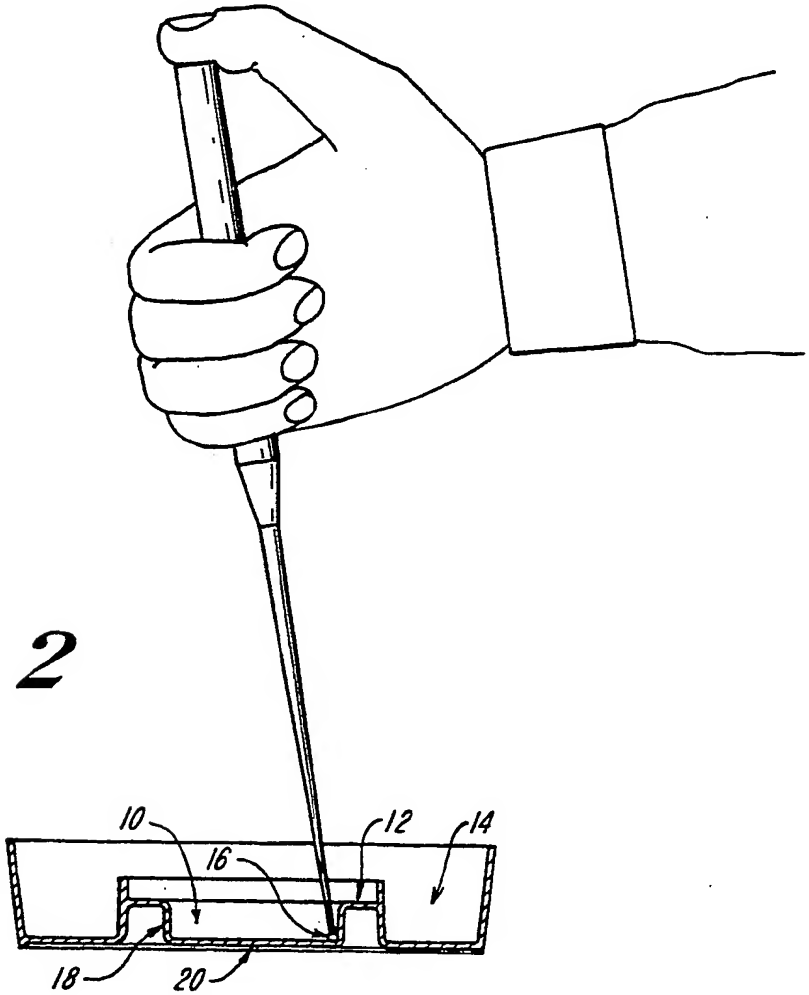

FIG. 2
(PRIOR ART)

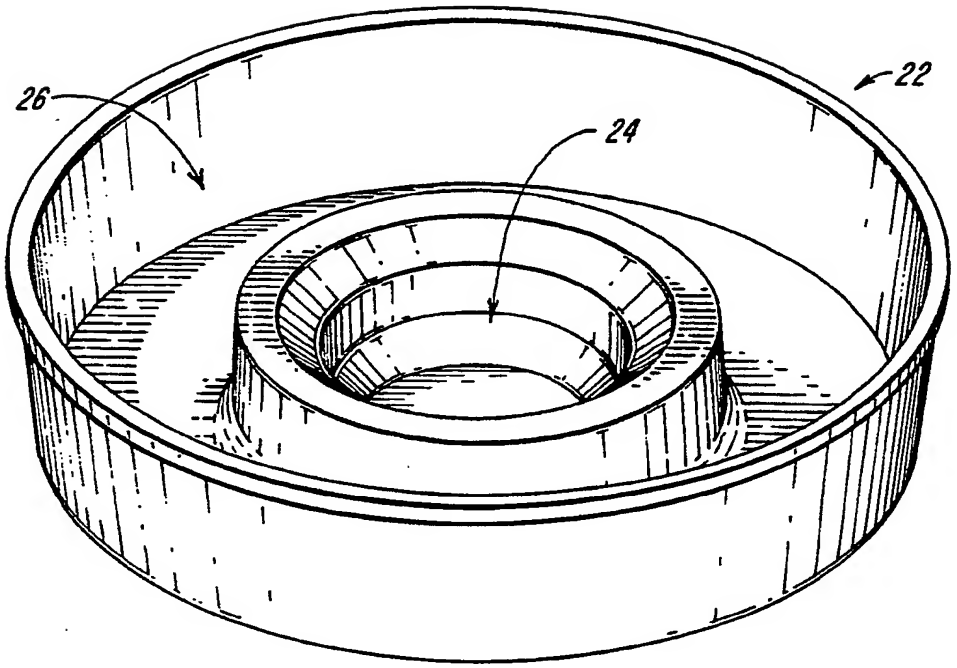

FIG. 3

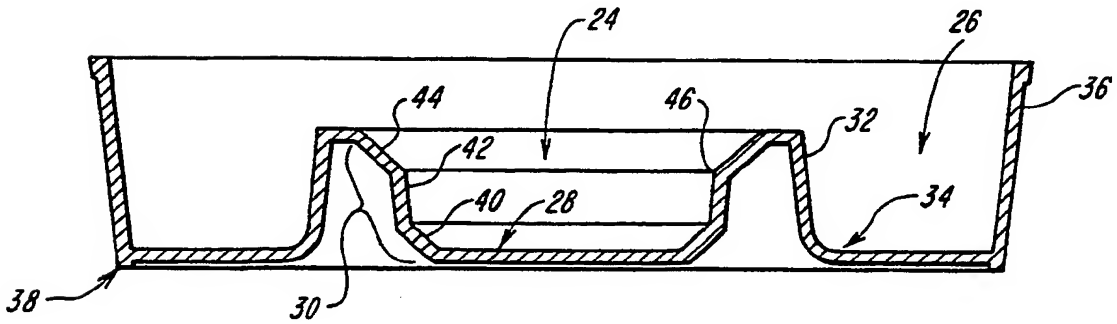

FIG. 4

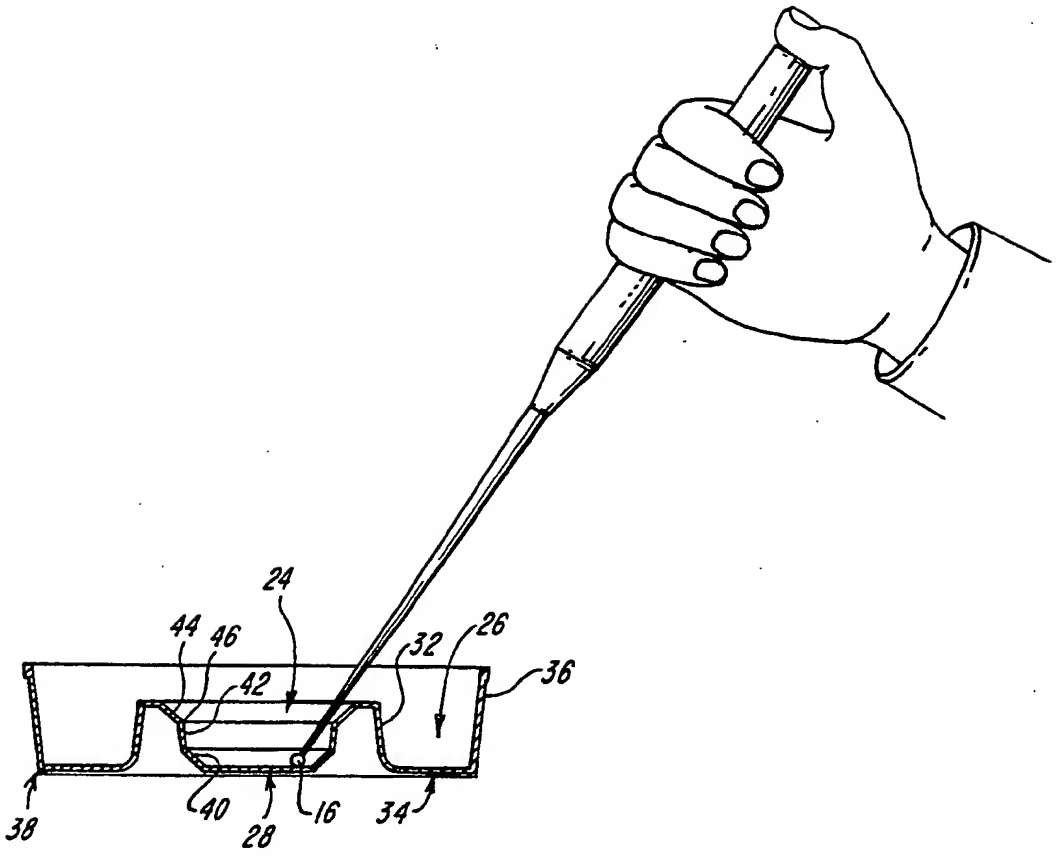

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06019

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12M 1/22, 1/20

U.S. Cl: 435/287, 297, 300, 301

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
US.Cl.	435/287, 297, 300, 301, 284; 604/33

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 5

APS (keywords: dish, ferlitization, culture) inverted microscope

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X/Y	US, A, 4,786,601 (B.E. Rothenberg) 22 November 1988, see Col. 3, lines 5-18.	1,5/19
Y	US, A, 4,255,522 (Fusenig et al.) 10 March 1981, see Col. 2, lines 24-30.	6,9,25,26
X/Y	US, A, 4,668,633 (J.R. Walton) 26 May 1987, see Fig. 1-3.	1/2-26
X/Y	US, A, 4,321,330 (Baker et al.) 04 April 1980, see Fig. 1-6.	1,5/2-4,6-26
Y	WO, A, 83/00047, (C.C. Reidel) 06 January 1983, see p.4, line 29-p.5, line 8 and Fig. 2 and 3.	2,6-9 10-15,17 18, 20-26
Y	JP, A, 2,051,977 (Terumo Corporation) 02 March 1987, see Fig. 1.	2,10-12
A	Cole-Parmer Catalog, "Plasticware" 1987-88, pp. 42, 93, 128.	1-26

* Special categories of cited documents: 15

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IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
03 January 1991	11 FEB 1991
International Searching Authority 1	**Signature of Authorized Officer 20**
ISA/US	Janelle Waack

Form PCT/ISA/210 (second sheet) (May 1986)